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FACTORS INFLUENCING INFECTION OF CEREALS BY THE ERGOT FUNGUS

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FACTORS INFLUENCING INFECTION OF CEREALS BY THE ERGOT FUNGUS

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

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OF MASTER OF SCIENCE

FACULTY OF AGRICULTURE

by

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FACTORS INFLUENCING INFECTION OF CEREALS BY THE ERGOT FUNGUS

S. G. Fushtey

INTRODUCTION

The fungus Claviceps purpurea (Fr.) Tul. which causes the Ergot disease of cereal crops and other grass plants is usually most important as a pathogen of rye. It has generally been of minor importance on barley, common wheat, and oats, but recently has been reported to occur rather abundantly on one of our new barley varieties, namely Montcalm. This has been one factor which has stimulated a new interest in the study of its pathogenic capabilities.

The ergot fungus is important from two different standpoints:

(1) it decreases the yield and quality of the products of affected crops

(2) it is poisonous and renders grains and grasses unfit as feed for livestock.

Considerable losses of crops, livestock, and even human life, have been experienced throughout the world over a long period of years.

Crop losses are represented not only by the number of grains which are visibly transformed into ergot bodies but also by a large number of blasted florets. Ergot has been particularly severe on rye and durum wheat in North Dakota. The disease is very common in Alberta and in certain seasons is highly destructive to rye and occasionally of some importance in barley and common wheat.

Sickness, and even death resulting from the consumption of ergot bodies in grains occurred as far back as the time of Caesar. Ergotism, a disease of humans and animals caused by the use of ergotized grain for food, has been, and in some regions still is, responsible for considerable losses. In 1922 an epidemic of ergotism in Spain and France cost, according to some estimates, approximately 40,000 lives (41). Reports of ergot poisoning in animals is not uncommon even today.

That conditions of environment play no small part in the development of the ergot disease is indicated by the fluctuation in severity of the disease from season to season and from place to place. Jones (22) emphasizes the importance of studying these conditions. He points out that the time has come when we should put relatively less stress upon the parasite as an

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independent organism and relatively more on the disease. As the first step in this he urges that increased attention be given to the study of the relation of the environment to disease inception and development. This relationship is abundantly illustrated by examples of studies of the ergot disease. Weniger (41) has noted that very little ergot occurs in dry seasons, whereas there may be abundant infection in wet seasons. Also, Hynes (21) concluded that the limiting factor to success in producing ergot commercially is the uncertainty of weather conditons when the rye comes into flower. Only in cooler, moister localities should such a project be undertaken.

In 1875, Wilson, as reported by Atanasoff (2), suggested that there may exist a relationship between the length of period during which the glumes are open and the amount of infection. Another interesting aspect was introduced by Stager who, as reported by Weniger (41) described the occurrence of biologic races within species of the ergot fungus.

The development of epiphytotics of ergot would therefore seem to be dependent on a combination of factors involving the host, the parasite, and the environment.

LIFE CYCLE OF CLAVICEPS PURPUREA

C. purpurea (Fr.) Tul. is an ascogenous fungus belonging to the family Hypocreaceae. It produces three distinct stages in its life cycle:

(1) The vegetative mycelium, which permeates and destroys the young ovaries of susceptible host plants and produces an asexual or conidial stage (the sphacelial stage) on the surface of the young fungus growth.

(2) The matured sclerotia or ergot bodies which are dormant or resting structures formed from the mycelium. These permit the pathogen to live through the winter or other unfavorable periods.

(3) The ascigerous or sexual stage, which is formed when the sclerotia germinate. This produces ascospores.

The first infections of each growing season usually originate from wind-blown or insect-borne ascospores. These reach the young ovary of an open flower, germinate, and the resulting hyphae invade the tissues of the ovary replacing it by a fungus growth which maintains the general shape of the ovary. The surface of this fungus body is channeled or convoluted and is covered with short conidiophores which produce enormous numbers of hyaline, ovate or sphaeroid conidia (Fig. 1, A). During this stage of development, there is a copious

[illegible] of [illegible] [illegible]

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secretion of "honeydew" which collects in droplets at the edges of the glumes and is teeming with these newly formed conidia. This sphacelial stage is said to be visited by insects which carry the conidia to other flowers and thus spread the fungus during the flowering period.

As growth progresses, the development of conidia gradually ceases, the mycelium becomes gradually compacted, the structure thickens and the superficial hyphae turn violet colored. This change progresses upwards until the entire sphacelial stage has disappeared and in its place is formed a horny, dark-colored sclerotium which projects out of the ear, (Fig. 1, B and C). These sclerotia either fall to the ground or are mingled with the harvested seed.

After a resting period, normally from fall to the following spring, the sclerotia which are lying on or in the soil germinate by the production of stromata, each consisting of a slender, reddish, pale-violet or whitish stalk surmounted by a reddish, flesh or fawn-colored, globular head, (Fig. 1, D). This stromatal head or sphaeridium is covered with minute elevations, the projecting ostioles of the perithecia, embedded in the periphery, (Fig. 1, E and F). The perithecia are filled with curved, hyaline asci each containing a bundle of eight slender, hyaline, septate, needle-shaped spores, (Fig. 1, G and H). The ripened ascospores are expelled from the asci, and if picked up by the wind or by insects and carried to open flowers of susceptible hosts may initiate the disease.

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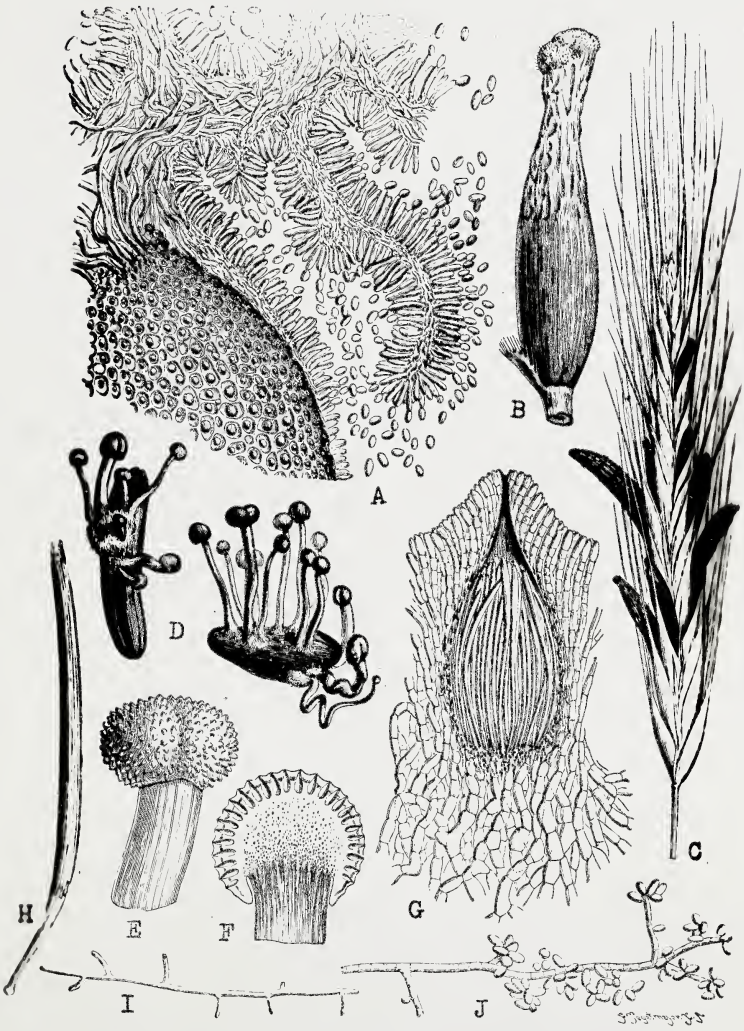

Fig. I. Stages in the Life Cycle of C. purpurea. (After Gaumann and Dodge).

A. Section of conidial layer.
B. Young sclerotium.
C. Mature sclerotia on rye.
D. Germinating sclerotia.
E. Perithecial head.
F. Section of perithecial head.
G. Section of perithecium.
H. Ascus.
I. Germinating ascospore.
J. Conidial hypha from culture.

SCOPE AND PURPOSE OF THE PRESENT STUDY

The present work was confined to a study of the conidial stage, the repeating or multiplying stage, of the ergot fungus. Not only is it of major importance in relation to the development of the disease but it can be produced at will in artificial culture and hence lends itself to investigation in the laboratory. In nature, conidia are found in abundance in the honeydew which is secreted in the early stages of disease development in the host. This may multiply rapidly under favorable conditions, and may bring about severe outbreaks from a comparatively small amount of initial inoculum. Hence it is important to study the influence of the environment on the conidial stage and on infection produced by it.

The present study was undertaken for the purpose of improving techniques of inoculating host plants so that these might be available for testing ergot resistance of the cereal varieties and hybrids.

The work was divided into two related but separate fields of study and is reported under two main sections:

Part I - Germination of Conidia.

Part II - Infection of the Host.

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The work was divided into two related but separate fields of study and is reported under two main sections:

Part I - Germination of Conidia.

Part II - Infection of the Host.

Physical, chemical, and biological factors were considered with respect to spore germination as well as disease inception.

PART I

FACTORS INFLUENCING GERMINATION OF CONIDIA

Introduction

Since all fungus diseases must begin with infection and since germination of the spore often precedes infection, knowledge of the conditions which favor or inhibit spore germination is important.

The factors influencing germination of fungus spores are usually divided into two types: hereditary or internal, and environmental or external. Internal factors include maturity, longevity, dormancy, and vitality of spores. External factors, which constitute the group of factors involved in the present study, include moisture, temperature, pH, light, and kind and concentration of nutrients.

CHAPTER I

FACTORS CONTRIBUTING DIRECTLY TO CHANGE

Introduction

Since all human discoveries have their roots also influenced but since consideration of the means other than technical innovations of the conditions which cause or induce a change are inadvertently ignored.

The Western Industrial Revolution of course spring has usually limited into two broad categories as technical and environmental or material. Internal factors include ability, longevity, resources, and stability of society. Material factors, which constitute the group of factors involved in the present study, include population, transportation, [illegible], and [illegible] and characteristics of societies.

Numerous observations have been made on the factors influencing spore germination in many different fungi but information of this nature for the fungus C. purpurea is practically non-existent. McCrea (28) conducted careful experiments to determine the effects of variations of environment on the fungus in saprophytic culture but no spore germination studies were reported. Reference to the work of others on spore germination is, therefore, necessarily confined to studies of fungi other than C. purpurea.

Since the present study deals with external factors brief mention of internal factors is made here mainly to illustrate some of the basic properties of spores in general, and to relate these properties to ergot conidia.

"Dormancy" is a characteristic exhibited by spores of many fungi. Zygospores and oospores of phycomycetes, chlamydospores of smut fungi, and teliospores of rust-fungi require a period of dormancy before they are able to germinate. Davis (9) stored spores of Ustilago striaeformis in the laboratory for about 240 days before he could secure germination. Ergot conidia apparently require no such dormancy period. This could be expected from the nature of field infection which occurs very soon after honeydew has been produced.

"Longevity" of spores varies with different fungi. As far back as 1872, Brefeld, as reported by Wolf (44) noted that chlamydospores of Tilletia tritici, when kept dry in the herbarium for 8½ years were still germinable. Those of Urocystis cepulae are reported to remain viable in the soil for at least 5 years. Smith (37) secured germination of spores of Myxomycetes from herbarium specimens 5 to 32 years after collection. On the other hand, conidial forms usually lose their ability to germinate much more rapidly. According to Aderhold, as reported by Doran (12), the conidia of Venturia inaequalis retain their viability no longer than 8 weeks. Stager, as reported by Atanasoff (2), demonstrated that conidia of Claviceps purpurea on immature sclerotia, stored in a dry condition, retained their viability and pathogenicity even after 10 months.

"Maturity" of spores is very important in some species. Doran (12) reported that when conidia of V. inaequalis were obtained by brushing apple scab lesions, only 25% as many conidia germinated as when conidia were obtained by washing with a stream of water. He explained that a large number of immature spores were obtained by the brush method. No references are available regarding germinability of immature conidia of C. purpurea but results from studies reported here indicate that, other factors being favorable, good germination can be obtained

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[illegible] for [illegible] [illegible] [illegible] [illegible]. [illegible] [illegible]
[illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible]

with spores from cultures just beginning to sporulate as well as from those which are several weeks old. Furthermore, no differences in germinability were observed between spores obtained by mechanical removal from the mycelial mat and those removed by water alone. Maturity does not seem to be a limiting factor for germination of conidia of the ergot fungus, but a more comprehensive study of this factor is required to confirm this observation.

Regarding external factors, a detailed discussion is presented in the sections which follow. Each of the factors is treated separately, the literature review, experimental data, and other pertinent material being dealt with under the respective factors concerned.

Experimental Methods for Spore Germination Studies

The hanging-drop technique initiated by Hoffman in 1860 and described by Duggar (15) was employed for most of the germination studies. Van Tieghem cells, about 2 cm. in diameter and 1 cm. deep were used throughout. These were cemented to glass slides by a mixture of refined beeswax and vaseline.

also [illegible] [illegible] to [illegible] as well as from [illegible] [illegible]. [illegible] differences in [illegible] [illegible] [illegible] obtained by [illegible] [illegible] [illegible]

To maintain a high humidity within the cell, a few drops of the same solution used in the hanging-drop was added to the bottom of the cell before the covers were fixed in place. Each slide held two such cells and was enclosed in an ordinary Petri dish for protection while handling.

Extreme care was taken in cleaning glassware to be used in these experiments. The dishes, cells, slides, pippetes, etc. were all thoroughly cleaned in acid dichromate cleaning solution, washed thoroughly in distilled water and allowed to soak in fresh distilled water for at least 12 hours before use. The glass cells, and cover slips in particular, were boiled in the cleaning solution, washed repeatedly in distilled water to remove all visible traces of the cleaning agent, boiled for at least 15 minutes in a fresh change of water and finally stored in fresh distilled water for at least 12 hours before use.

In spite of all these precautions very irregular results were obtained in replicates of some of the treatments. Duggar (15) experienced similar irregularities in his work when using conditions which ordinarily do not cause abundant germination. He explained that volatile or soluble substances used besides the medium employed may have undesirable effects. Even the purest vaseline may have an effect on sensitive forms. He therefore used a modified method for cultures where the full

nutrient media were not employed. The cells were used in Petri dishes. Filter paper with holes made for the insertion of the cells, to secure them against movement, was placed in the bottom of the dishes. The cover slips were laid on the cells without vaseline. All cultures were kept in moist chambers.

In the later experiments of the present study an adaptation of Duggar's modified method was employed. The filter paper in each dish was soaked with sterile distilled water to provide a moist atmosphere within the dish. Cover slips were fixed to the cells by touching the outside edge of the glass ring with a drop of water which spread rapidly around the ground surface of the glass cell and secured the covers against sliding when handled. This method, in addition to eliminating the possible interference by volatile materials in the cementing substances, also eliminated all the labour involved in applying the cement substances and in removing them before the cells could be used again.

Spores were obtained from cultures grown on test-tube slants of Leonian's agar medium as given by Rawlins (34). Three per cent agar was used to provide a lasting, firm medium, which did not break up when spores were being removed. The cultures were grown in the laboratory at room temperatures and

spores were harvested as needed from cultures 10 to 20 days old. The spores were removed by washing with small amounts of sterile distilled water, sometimes using a very flexible wire loop to disturb the surface of the culture and thus release additional spores. Small amounts of this heavy spore suspension were then added to the test media, drops of which were transferred to the clean, dry cover slips made ready for the preparation of the Van Tieghem hanging-drop cultures. These cover slips were then inverted, placed on the glass rings and fixed in place as described in the preceding paragraph. Before the cultures were set aside for incubation they were examined microscopically to make sure that the concentration was sufficient to permit a count of at least 100 spores per culture but not so high as to interfere with ready observation.

Effect of Temperature on Germination of Conidia

One of the most important external factors bearing upon the germination of fungus spores is the temperature relation. A number of fungi have been studied with respect to the influence of temperature on germination of spores. For

example, Doran (12) assembled in tabular form, data on the influence of temperature on spore germination of a variety of plant pathogenic fungi. His reports indicate that the average cardinal (minimum, optimum, and maximum) temperatures for Phycomycetes are 1.2°C., 18°C., and 26.1°C. - a range of 25.0 degrees; and for conidia of V. inaequalis: 3°C., 15°C., and 31°C. - a range of 28.0 degrees. Germination of conidia of V. inaequalis dropped rapidly below 12°C. and above 18°C.

Regarding C. purpurea, McCrea (28) reported that the growth of this fungus in agar culture occurs over a wide range (18° - 30°C.) but is slow and scanty at 10°C., and is completely inhibited at 37°C., although growth is resumed if the culture is returned to optimum levels. These reactions do not necessarily hold for germination of spores, for according to Doran (12), there is no reason to believe that the optimum temperature for the growth of a fungus is the same as the optimum temperature for spore germination. He maintained that each should be studied separately.

Further reference to temperature effects was made by Hershenson and Plaut (20) who reported on inoculation of rye with spores of C. purpurea. They pointed out that the optimum temperature for infection in the field is 20° - 30°C. and that very little infection occurs at temperatures outside

of this range. It is expected that the conditions for germination of spores would more nearly approach those for infection since spore germination is prerequisite to infection. Nevertheless, since no specific data on the relation of temperature to germination of Claviceps conidia has been recorded to date, the study here reported was undertaken.

Experimental

Spores were added to hanging drops of sterile redistilled water, the cultures being prepared as described in the section on experimental procedure, using the adaptation of Duggar's methods. An inadequate number of glass rings made it necessary to limit the range of temperatures tested in one experiment so that the first set of experiments covered the range 10° to 25°C. and was followed with another at the range 20° to 40°C.

Considerable difficulty was encountered in obtaining reproducible results when using spores from artificial culture. Spores in a large number of cells did not germinate at all when those in other cells, under the same conditions, germinated quite well. Repetition of these experiments yielded similar results.

It was suspected that probably minute residues of cleaning solution or other impurities on the glass may have been responsible for such behaviour and that the precautions taken in rinsing the glassware may have been inadequate. The glass rings and cover slips were therefore soaked for an extra 24 hours in several changes of distilled water before use. Results still failed to improve. In order to secure comparable replicates, 8 cells were prepared for each treatment, only 4 showing best germination eventually being used for observations.

Finally, a dilution of conidia from honeydew on immature sclerotia, which had been stored in the refrigerator at about 20°C. for 2 months, was used as a source of spore material.

The results are reported in Table 1 under the following headings:

1. Spores from artificial culture at 10° to 25°C.
2. Spores from artificial culture at 20° to 40°C.
3. Spores from honeydew at 10° to 25°C.

One hundred to 200 spores were counted in each of the cells selected to represent the particular temperature level. The average germination percentages in Table 1 are therefore based on counts of at least 500 spores.

Table 1

Effect of Temperature on Germination of Conidia of *C. purpurea*

Source of spores	Temp. °C.	Average percentage of germination after different periods (hrs.) of incubation					
		18	24	42	48	60	96
1. Artificial culture	10		0		25.5		87.3
	15		0		27.8		89.1
	20		49.7		79.1		100.0*
	25		90.6		96.3		100.0*
2. Artificial culture	20	tr		41.1		44.0	
	25	tr		24.1		25.1	
	30	tr		3.2		3.0	
	35	0		0		0	
	40	0		0		0	
3. Honeydew	10	0	0	4.5	4.2		
	15	3.3	3.2	13.7	16.9		
	20	44.9	75.7	97.8	97.4		
	25	85.5	98.4	98.7	100.0		

* Only a few ungerminated spores found in each culture.

[illegible]

[illegible]

Because little was known regarding the time required for germination of the conidia, it was decided that observations be taken at intervals beginning at 18 hours and ending at 96 hours. The data indicate that 48 hours would be the best period of incubation at which to make spore counts in subsequent experiments.

An interesting observation was that spores from naturally produced honeydew germinated better than those from artificial culture. Furthermore, development of germ tubes from the former was much more rapid than from the latter. For instance, in 48 hours at 25°C. the germ tubes of the natural conidia were too long to permit counting, whereas, those of spores from artificial culture were not more than 100 microns long, and could be counted without difficulty. It is possible that spores from natural sources are more vigorous or that spores from artificial culture are much more sensitive to some undetermined factors which tend to inhibit germination under the conditions provided.

In order to avoid the necessity of having to draw conclusions from data as variable as those presented in Table 1, a complete experiment covering the range 10° to 40°C. was set up once a sufficient number of glass rings was obtained. An attempt was made to eliminate erratic germination such as occurred in some of the previous experiments by using spores from honeydew, produced on rye in the greenhouse during some of the infection studies.

Notes on germination and germ tube length were taken after 48 hours of incubation. The results are presented in the graph in Fig. II. The average germ tube length was determined by selecting that length which seemed to predominate in the particular treatment and measuring it by means of an ocular micrometer.

It is interesting to note that germination and growth of germ tubes varied together and that both fell off much more rapidly at temperatures higher than the optimum than at temperatures below optimum. These observations indicate that the two processes are affected similarly by temperature.

According to the graph, the optimum temperature for both germination and growth is 25°C. Actually, the series of experiments preceding that represented by the graph indicate that the optimum is somewhere between 20° and 25°C., probably nearer 25° than 20°, but the data thus far obtained do not permit a more accurate estimate. Similarly, the maximum temperature is between 30° and 35°C. and the minimum probably between 5° and 10°C.

To determine the influence of prolonged exposure to temperatures beyond the limits for germination, the cultures which had been incubated for 48 hours at 35° and 40°C., as well as spores in diluted honeydew which had been kept in ice for 48 hours, were incubated at 25°C. for a similar period.

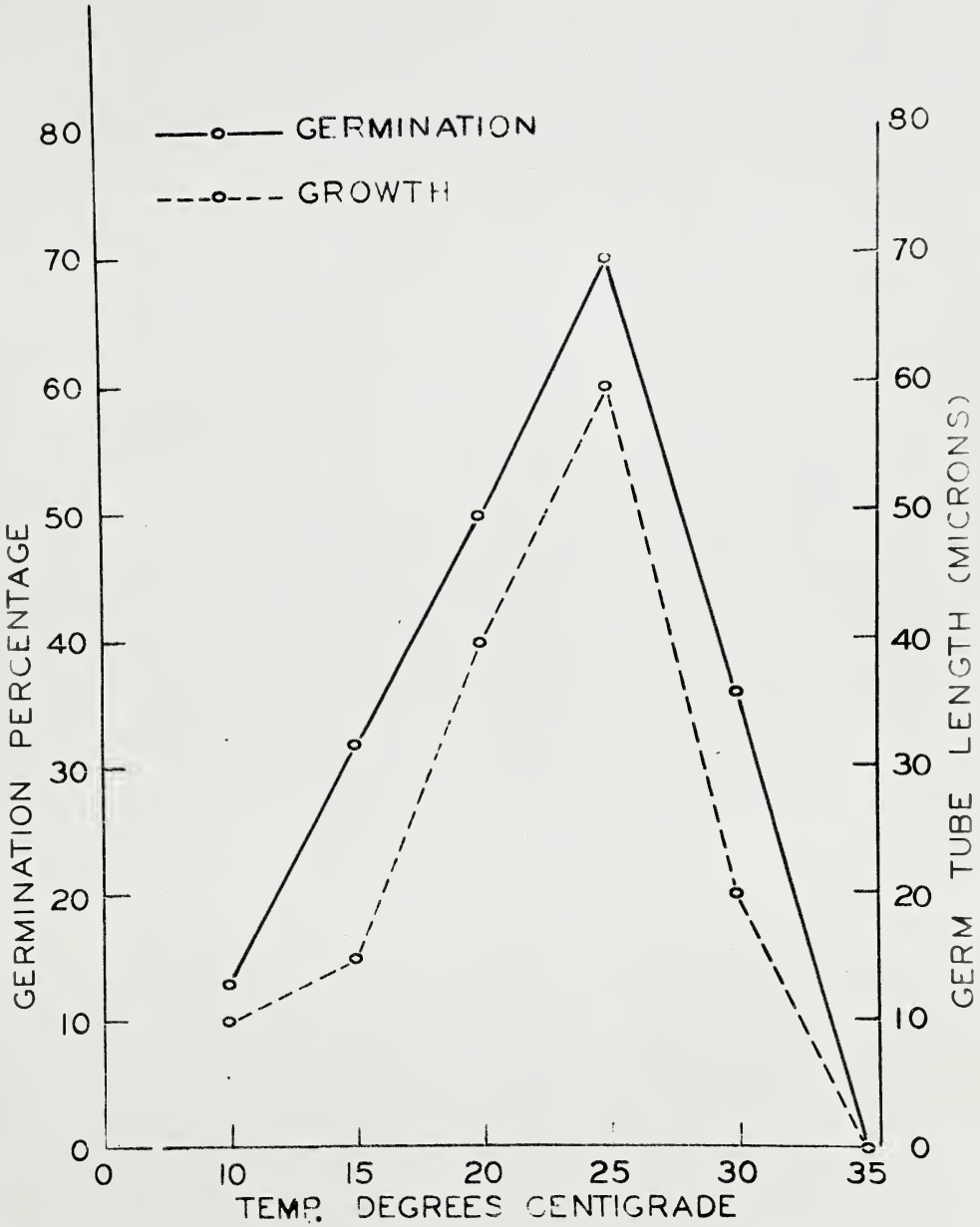

Fig. II. Effect of Temperature upon the Percentage of Germination and Germ Tube Length of Conidia of C. purpurea.

The spores which had been frozen germinated quite readily while those which had been kept at the high temperatures did not germinate.

Apparently, prolonged exposure to temperatures of 35°C. and higher tend to permanently inactivate conidia of the ergot fungus.

Effect of Moisture on Germination of Conidia

Water, either in liquid or vapour form, has long been recognized as essential to the germination of spores of fungi. An examination of the literature reveals that the moisture requirements for germination is not uniform for all spores. Doran (12) recorded that conidia of *Sclerotinia fructigena* and *Peronospora pygmea* germinate only when in direct contact with water, but that conidia of *Alternaria solani* and *V. inaequalis* may germinate in moist air. However there is considerable variation in the moisture requirements of the different species of those fungi that do germinate in moist air. Brodie and Neufeld (6) found that conidia of *Erisiphe polygoni* germinated through a range of relative humidities from approximately zero to 100%. On the other hand, Clayton (7)

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Effect of moisture on germination of conidia

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found that conidia and ascospores of V. inaequalis germinated on dry glass only if the relative humidity was 99 to 100%.

A number of workers have noted the influence of moisture conditions in the field upon the process of infection by the ergot fungus but no data are available on the moisture requirements for germination of its spores. The work of Hershenson and Plaut (20), Hynes (21), and others, has indicated that a high level of humidity is required for infection to occur in the field. Ready germination of spores is undoubtedly important in the initiation of infection and it seems likely that moisture is a controlling factor in this respect. To determine the extent to which moisture can be a controlling factor, the present study on the germination of conidia was undertaken.

Experimental

Saturated aqueous salt solutions, aqueous sugar solutions, and redistilled water were used to control the relative humidity within sealed 1-quart glass fruit jars, as described by Clayton (7). The following materials at the concentrations given and held at 20°C. provided theoretical relative humidities at the four levels indicated.

Material used	Concentration of solution	Theoretical relative humidity
Redistilled water	-	100%
Sucrose	0.50 molal	99%
$K_2 HPO_4$	saturated	92%
$(NH_4)_2 SO_4$	saturated	81%

Spore suspensions were obtained from artificial culture by the flooding method and were streaked onto clean glass cover slips by means of a fine camel's-hair brush. These preparations were dried by exposing them to the open air of the laboratory for about 5 minutes. Each was then attached with paraffin to one end of a short glass rod. The other end of the rod was heated and imbedded in paraffin inside of one of 4 Van Tieghem rings that had been sealed with paraffin to the under surface of a glass top of a humidity chamber. This top was then substituted for one on a chamber of the desired relative humidity. Two chambers, each containing 4 preparations, were used for each level of humidity. The chambers were kept in a temperature cabinet regulated at $20^\circ \pm 1^\circ C$. This variation may have caused a slight fluctuation of humidities since the conditions, as given by Clayton (7), required an accuracy of $20^\circ C. \pm 0.02^\circ C.$, but the results of the

[illegible]

[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

experiment indicated that, in this particular case, the effects of such variations would be unimportant.

Observations were made at 24, 48, and 72 hours. Two preparations, one from each chamber, were removed at each interval, leaving four for the final reading. The preparations were removed from the glass rods, and inverted on slides covered with a film of water, for microscopic examination. Samples of the original spore suspension used in the above preparations were also incubated in hanging-drop cultures for comparative purposes.

No germination was observed in any of the dry glass preparations. Some germination occurred in 48 hours in the hanging-drop cultures, but germination was low even at 72 hours.

Following examination, the dry glass cultures from the different chambers were flooded with a drop of water and prepared in the form of hanging-drop cultures to determine the germinability of these spores which had been dry for 72 hours. No germination occurred in any of the cells after 48 hours of incubation. The spores were apparently inactivated by the conditions of the previous experiment.

The experiment was repeated using diluted honeydew for spore material. Results were the same as those of the previous experiment except that spores in hanging-drop cultures

germinated very well (nearly 100% in some cells) and there was some germination of spores at the 100% relative humidity where small amounts of moisture had condensed on the surface of the glass carriers. No evidence of germination was found on any of the dry carriers after 72 hours incubation in the humidity chambers, or later, when these same carriers were incubated at 25°C. as hanging-drop cultures.

The results indicate that the conidia of C. purpurea require contact with liquid water for germination.

Effect of Nutrition on Germination of Conidia

Some spores do not require external nutritive elements for germination. Other species are dependent upon the presence of sugars and minerals. Lin (27) reported that the conidia of Glomerella cingulata require carbon, magnesium, nitrogen, and phosphorus for favorable germination. Cooper (8) found that various amino acids, vitamins, and other substances induced germination of Colletotrichum gloeosporioides. Wolf (44) mentions that in general, where germination is difficult in water

alone, an attempt should be made to approximate natural conditions of germination.

Preliminary observations in the present study indicated that spores from artificial agar cultures of C. purpurea do germinate, though poorly, in pure water, whereas, naturally produced spores in honeydew diluted in pure water germinate abundantly. This information, along with the report by Kirchoff (23) that undiluted honeydew corresponded to a 2.33 molar sugar solution, suggested that sugars may be required for abundant germination of conidia of C. purpurea. Experiments were therefore conducted to determine the effects of various sugars on spore germination.

Materials and Methods

The sugars tested were xylose, maltose, lactose, sucrose, glucose, fructose, and galactose. These were dissolved in distilled water to make 1% solutions. The solutions were sterilized in an autoclave for 20 minutes at 15 pounds pressure before being used. Van Tieghem cells were employed in the germination tests which were conducted at room temperature (20° - 25°C.) A control, using pure redistilled water, was always included. The spores used were in all cases obtained from the surface of agar test-tube slant cultures by the flooding method.

Experimental

Four Van Tieghem hanging-drop cultures were prepared for each sugar. Notes were taken after 36 hours incubation at room temperature. The results from the first few experiments were so variable that a number of experiments using as many cultures as possible were considered necessary before any general conclusions could be drawn. The experiment was therefore repeated 5 times using 6 hanging-drop preparations for each sugar tested. At least 100 spores were counted in each drop. The results are summarized in Table 2 and are expressed in terms of percentage germination, each value being based on a total of 400 to 600 spores counted.

Table 2

Influence of Sugars on the Germination of Conidia of *C. purpurea*

	Percentage germination					
	Experiment number					
Sugar used	1	2	3	4	5	Average
Fructose	2.2	56.7	25.8	78.8	24.1	27.5
Glucose	78.7	94.6	35.7	80.8	25.1	62.9
Lactose	70.2	92.0	33.7	68.2	46.3	62.1
Sucrose	22.0	92.0	41.8	68.8	50.5	55.0
Maltose	83.2	100.0	100.0	78.2	100.0	92.3
Galactose*	--	--	12.6	79.5	16.9	36.6
Xylose*	--	--	--	69.3	43.8	56.5
Water	7.4	57.8	24.3	53.4	6.0	21.3

* Not used in statistical analysis.

With variations as great as that found in fructose, which varied over a range of 76% between experiments, the average percentages of germination for the five experiments seemed highly unreliable. In order to provide some basis for discussion a statistical analysis of variance was carried out using the different experiments as replicates within one large experiment.

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[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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Analysis of Variance Table:

Variance due to	D.F.	T.S.	M.S.	f
Experiments	4	8496.27	2124.07	6.36**
Sugars	5	12148.26	2429.65	7.27**
Error	20	6679.04	333.95	
Total	29	27323.57		

** Highly significant.

M.S.D. for sugars = 22.1% germination.

(The percentage data were analyzed as presented, transformations being considered unnecessary when such highly significant results were obtained).

The high significance of the variance due to sugars and the value of 22.1% germination for the M.S.D. for sugars indicate that, of the sugars tested, all but fructose increased germination of the conidia, and maltose gave by far the highest germination.

The high significance of the variance due to replicates indicates the existence, from one experiment to the next, of some variable factor which had not been accounted

Analysis of variance table:

Variance ratio	[illegible]	[illegible]	[illegible]	[illegible]
Regression	[illegible]	[illegible]	[illegible]	[illegible]
Error	[illegible]	[illegible]	[illegible]	
Total	[illegible]	[illegible]		

[illegible] highly significant.

[illegible] for regression = [illegible].

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for in the conditions of the experiment. It is difficult to visualize what this factor may have been. The sugars used were always from the same lot, materials and methods were, as nearly as could be determined, the same for all experiments. The only known condition that could have varied was room temperature but, as indicated by a previous section of this project, temperature variation between the levels of 20° and 25°C. do not affect germination to any great extent. It may be, that conidia are very sensitive to some factor such as the presence of some foreign material in minute quantities and that these materials contaminated the cultures at one time or another. It may also be that bacterial activity inhibited germination of spores in some cases. Considerable bacterial development was observed in some of the maltose solution cultures and germination of spores in these was obviously inhibited. On the other hand, spores in cultures in which no bacteria could be detected, germinated very poorly also.

Nevertheless, it is demonstrated that sugars increase germination of conidia of C. purpurea and that maltose is outstanding in this respect.

Germination with Respect to Concentration of Sugar Medium

The undiluted honeydew, as reported by Kirchoff (23), corresponded to a 2.33 molar sugar solution. The millions of conidia suspended in this honeydew remain dormant, or at least do not germinate, as long as the honeydew remains undiluted. Once the honeydew is diluted the conidia germinate readily if subjected to favorable temperature conditions. The following experiment was carried out to determine the maximum sugar concentration that will permit germination of conidia and at the same time to give some idea of the extent to which honeydew must be diluted before germination will take place.

Maltose solutions were used in place of the honeydew sugar and spores from artificial culture were suspended in these solutions for germination tests. The following concentrations of maltose were prepared in distilled water: 2.0 molar, 1.0 molar, 0.5 molar, 0.25 molar, 0.1 molar, and 0.05 molar. A drop of a concentrated spore suspension was added to 10 ml. portions of each of the above solutions, mixed thoroughly, and single drop portions tested for germination in Van Tieghem hanging-drop cultures. Four cultures were prepared for each concentration. They were examined at the end of 36 hours incubation in humidity chambers at room temperature.

Good germination (70 - 80%) took place in the 0.05 and 0.1 molar solutions. About 20% of the spores germinated in the 0.25 molar solution, and only a trace germinated in the 0.5 molar one. Hanging drops of both of the last two solutions increased in size during germination, indicating that further dilution had taken place in the humid atmosphere of the chambers. No germination occurred in the 1.0 and 2.0 molar solutions.

These results indicate that, if natural honeydew is 2.33 molar, it is probable that at least tenfold dilution is necessary before any of the spores can germinate. For good germination this dilution should be doubled i.e. the 2.33 molar sugar solution should be diluted 20 times to give an approximately 0.1 molar solution (approximately 3.6% solution of maltose). Later experiments showed that no measurable difference in germination occurred in maltose solutions of 1 to 5% concentrations.

Since maltose gave by far the best germination of conidia, and since germination was not improved by increasing the concentration of maltose solution above 1%, the 1% maltose solution was adopted as the standard medium for spore suspensions for the inoculation work.

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Effect of Light on Germination of Conidia

Opinions on the effect of light on spore germination vary considerably. According to DeBary, as reported by Doran (12), light inhibits the germination of spores of Oomycetes. Melhus (30) found that light does not interfere with the germination of the conidia of *Phytophthora infestans*. Doran (12) tested the effect of sunlight on the germination of spores of *A. solani* and of conidia of *S. fructigena*. He found that the spores of these fungi germinate quite as well in sunlight as they do in darkness, provided that the conditions of temperature and moisture remain near the optimum. The following investigation was carried out to determine the relationship of light and dark to the germination of conidia of *C. purpurea*.

Experimental

Van Tieghem hanging-drop cultures of the conidia were prepared in the usual manner. For the experiment 12 Petri-dish humidity chambers, each containing two hanging-drop cultures were used. Four of these chambers were enclosed

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in a hard, black paper box to exclude all light. The preparations were then all placed on a laboratory bench exposed to the light from windows along the west wall of the laboratory. They were all moved in the afternoon in order that the temperature would not be raised too high by the direct rays of the afternoon sun. In the evening, four of the chambers were moved to another room so that they might be exposed to continuous light for the entire incubation period. Light was provided by a double-tube flourescent fixture suspended at a distance of about 4 feet from the top of the table on which the cultures were placed. They were returned to the daylight in the morning.

Notes were taken after 24 and 36 hours of incubation. Since the cultures were prepared at 9:00 a.m. the uncovered cultures had been exposed to two periods of daylight by the time of the final examination. The experiment was performed in mid-February with approximately 9 hours of daylight per day so that the total period of light and dark for the set of cultures which was not subjected to artificial lighting at night, was approximately the same, i.e. approximately 18 hours of daylight and 18 hours of darkness.

The results of germination counts at the end of the 36 hour period are shown in Table 3 and are expressed in percentage germination based on a total count of at least 200

spores per replicate. Each chamber, consisting of 2 cultures, was considered a single replicate.

Table 3

Percentage Germination in Light and Dark

Treatment	Replicates 1	2	3	4	Average
Continuous light	55.6	72.3	63.0	59.2	62.5
Light and dark	66.8	69.2	79.2	70.1	71.4
Continuous dark	67.1	73.2	60.8	68.9	67.5

The average percentages for the different treatments show very little variation of spore germination in light as compared to that in the dark. A statistical analysis showed that the variance due to light was not significant. This indicates that, at least under the conditions of this experiment, the conidia of C. purpurea germinate equally well in light, in darkness, and in alternating light and dark.

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[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

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Effect of pH on Germination of Conidia

The reaction to pH has been determined for many species of fungi. Usually the pH range for germination is quite wide. Most fungi germinate and develop best in acid media. However, Webb, as reported by Wolf (44), found spores of Colletotrichum gossypii to germinate best within the alkaline range. The optimum pH for germination of most slime mold spores, as reported by Smart (36), was 4.5 to 7.0. Conidia of Botritis cineria germinated over a range of pH from 1.6 to 9.6, depending on the nutrient in the medium, but the average range was 4.0 to 7.0 (36).

Experimental

I. In a preliminary experiment various levels of pH were obtained by the use of buffer solutions according to formulae of Gortner (16). The following solutions were prepared, all diluted to 20 cc. volumes to obtain the indicated pH:

[illegible] of [illegible] on [illegible]

[illegible]

[illegible]

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pH 3.0 - 50 cc. M/5 KH Phthalate + 46.70 cc. M/5 HCl

pH 5.0 - 50 cc. M/5 KH Phthalate + 23.85 cc. M/5 NaOH

ph 7.0 - 50 cc. M/5 KH_2PO_4 + 29.63 cc. M/5 NaOH

pH 9.0 - 50 cc. M/5 H_3BO_3 + 50 cc. M/5 KCl + 21.30 cc. M/5 NaOH

Five 10 cc. portions of each were transferred to glass vials and sterilized in a steam sterilizer for 20 minutes at 15 pounds pressure. One drop of a concentrated suspension of conidia from agar culture was then added to each vial and the lot incubated at room temperature for 24 hours.

At the end of the incubation period the mixtures were stirred and drop portions mounted on glass slides for examination. Four drops from each vial were examined and an average of 20 spores were observed in each drop. The percentages of germination, based on about 400 spores counted, were as follows:

pH 3 - none

pH 5 - 35.7

pH 7 - 19.2

pH 9 - 36.2

The levels of pH were checked colorimetrically at the end of the experiment and all solutions remained unchanged except for pH_9 which changed to 7.6. It was suspected that sterilization may have been responsible for this change. If so, little information can be obtained from the above results except that no germination occurred at pH 3.0. The low germination at pH 7.0 could not have been caused by the difference in reaction since much higher germination occurred at both pH 5 and 7.6.

II. In a later experiment sterile distilled water was used as the medium and was adjusted to the desired pH with dilute solutions of HCl and NaOH using an external electrode adaptation of the Beckman pH meter. One hundred cc. volumes in 200 cc. flasks were adjusted to the following reactions: pH 3, 4, 5, 6, 7, 8, 9, 10, and 11. Five ml. portions were transferred to Syracuse dishes (4 per treatment) and a drop of spore suspension from natural honeydew was mixed into the contents of each dish. Van Tieghem hanging-drops were also prepared for these mixtures. Both types of preparations were incubated at room temperatures for 24 hours. Germination counts were then made by direct microscopic examination, about 500 spores being counted for each treatment. The results are shown in Table 4.

Table 4

Percentage Germination of Conidia at Different Levels of pH

Adjusted pH	Van Tieghem cells	Syracuse dishes	pH at the end of the experiment
3.0	0.0	13.0	3.1
4.0	40.5	13.8	5.8
5.0	39.0	28.5	6.1
6.0	38.5	33.5	6.6
7.0	57.1	38.5	7.0
8.0	51.8	44.7	7.2
9.0	36.6	35.4	6.6
10.0	7.6*	9.1*	6.6
11.0	0.0	0.0	7.2

* Germ tubes small and distorted.

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[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
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[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

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The four replicates of each of the Syracuse dish solutions were bulked at the end of the experiment and the reactions again determined by the Beckman pH meter. The results are recorded in the last column of Table 4.

The change in pH here took place after the spores had been introduced to the medium since pH adjustment was not made until very shortly before the spores were added. Because the solutions were not buffered, it is likely that atmospheric CO_2 may have been responsible for lowering pH of the solutions on the basic side of neutrality but such could not be the case on the acid side. The acid solutions also tended to approach neutrality, but no satisfactory explanation for this can be provided on the basis of the work so far.

In spite of these changes, it is probable that the conidia had actually been exposed to the original pH during at least the initial period of germination. The experiment, although not accurately controlled, did give results which may indicate the approximate effect of pH on the germination of the conidia of C. purpurea. It would appear from the results that conidia of this fungus will germinate in distilled water over a range of pH from 4 to 10 but a range of 4 to 9 would be a safer estimate since germination was much inhibited at pH 10.

Lack of stability in the reaction of the preparations used in this experiment suggests that the results should be checked by further work, using strongly buffered solutions.

Discussion

Prior to the studies reported here, no information was available on the effect of environmental factors on the germination of the conidia of C. purpurea. The present studies have shown that temperature has a marked influence on the percentage germination of ergot conidia even if germination does occur over a comparatively wide range of temperature conditions. Germination was best at 20° to 25°C. with the upper and lower limits for germination being within the ranges of 30° to 35°C. and 5° to 10°C. respectively. Prolonged exposure of the conidia at 35°C. and higher resulted in permanent loss of germinability. These relationships indicate that the disease is likely to be of most importance in temperate climates.

The failure of the conidia of the ergot fungus to germinate in atmospheres of high relative humidities was rather

surprising in view of the fact that infection sometimes occurs in comparatively dry climates. Nevertheless, numerous workers have reported that moisture constitutes one of the major environmental influences on the inception of the ergot disease in crops. The occurrence of the disease in comparatively dry climates may be due to the effect of some other factor such as temperature which may influence conditions of humidity as well.

The nutritive requirements for germination of the conidia did not seem to be very great since germination occurred in pure, redistilled water. Nevertheless, the observation that conidia in diluted honeydew germinated much better than those collected from agar cultures and suspended in pure water, indicated that the honeydew may provide some stimulatory substances. Solutions of various sugars, and especially maltose, were shown to have similar stimulatory effects. Experiments to determine the concentration of sugar solutions that will induce germination of conidia indicated that concentrations up to 0.1 molar solutions are more favorable than higher concentrations and that natural honeydew needs to be diluted at least tenfold before any of the conidia can germinate. This fact that, as has been demonstrated, honeydew must be highly diluted before conidia can germinate suggests an important role of liquid moisture in the successful development of the pathogen at this stage.

In nature, spores of many fungi have been reported to germinate at night only. The present studies showed that, under controlled laboratory conditions, ergot conidia germinated equally well in light as in darkness. The ability of these conidia to germinate in darkness may probably explain the occurrence of the ergot disease in comparatively warm, dry climates. During the night the conditions of temperature and moisture nearer to the optimum are likely to prevail for a longer time so that conidia may germinate at night when daytime conditions are unfavorable.

Studies on the influence of pH on germination of ergot conidia did not yield conclusive results but indications were that the conidia will germinate within a range from pH 4.0 to 9.0.

PART II

FACTORS INFLUENCING INFECTION

Introduction

The production of disease by a pathogen involves three important processes in the following sequence: inoculation, incubation, and infection. The study of spore germination in vitro yields valuable information but the relation of the pathogen to the host itself is the critical relationship.

C. purpurea has been the subject of extensive study in the past both because of its destructive properties as a pathogen and because of the commercial value of the ergot sclerotia. During the first world war the price of ergot in the United States reached a high of five dollars a pound. This induced workers such as Bonns (4) to attempt the production of artificial sclerotia. Although the fungus, as a pathogen

of grasses and cereal crops, has been the cause of heavy losses in crops, animal and human life, most of the research on infection with C. purpurea has been done with the aim of finding methods of producing the valuable ergot sclerotia. Nevertheless, much has been learned in these studies that can be used by plant pathologists and other workers in the search for methods of control.

Literature

In 1921 McFarland (29) inoculated rye and wheat with Claviceps conidia from several native grasses and succeeded in producing infection and in inducing the development of sclerotia. Later Hecke (18) infected flowering rye with ascospores from ripe ascigerous fructifications, then, by removing the honeydew produced, suspending it in water and spraying it on other rye plants during the flowering stage, he was able to develop extensive infections. No details of his methods were given but he reported an average of 4.5 sclerotia per ear and suggested that cultivation of ergot appeared well worthwhile. Regarding host range of the pathogen, Stager (38) explained the rare occurrence of C. purpurea on wheat as being

the result of the brief and irregular opening of the glumes, the susceptible parts of the flower being ordinarily protected by the closed glumes.

Kirchoff (23), in a very extensive discussion of investigations during the period of 1921 - 25, discussed several new biological and physiological aspects of the ergot fungus and its pathogenic development. His observations showed that the principal seat of infection is the point of insertion of the ovary and that spores germinating on the stigma produce long germ-tubes which encircle the exterior of the ovary and then penetrate the tissue at the most susceptible point, the base of the ovary.

Krebs (24) gave an exhaustive account of comparative studies on three species of Claviceps and also discussed factors influencing field cultivation of ergot. Further work on field cultivation was reported by Bekesy (3) who described the use of a machine fitted with hypodermic needles in inoculating rye for ergot production.

More recent contributors include Melville (31), Andrus (1), Hynes (21), Saha and Battacharjee (35), Lewis (25) and (26), Thomas and Ramakrishnan (39), Brentzel (5), Hershenson and Plaut (20), Most of these workers concerned themselves with improved methods of producing ergot sclerotia. Further reference to some of these contributions will be made in the reports which follow.

Materials and Methods for Infection Studies

Most of the inoculation studies were carried out with spring rye but other crops such as wheat, oats, and barley were also used in some of the experiments. These crops were sown in 8-inch pots in the greenhouse and reached a stage suitable for inoculation in approximately three months. Pots of spring rye were sown at monthly intervals so that a constant supply of plant material for inoculation studies was available once the first three months had elapsed.

A supply of conidia was obtained by growing *C. purpurea* on test-tube slants of hard Leonian's agar medium. Spores were produced abundantly in two to three weeks and were harvested as needed.

Inoculum consisted of a spore suspension prepared in a manner similar to that used in the spore germination studies but the surface of the agar slant culture was scraped with a flexible wire loop in order to release as many of the spores as possible into the suspension. Such a procedure could not be used in the spore germination studies because the bits of mycelium and medium released interfered with the controlled conditions in the hanging-drop preparations.

Inoculation was performed by two methods:

1. The spore suspension was sprayed onto the florets by means of a DeVilbiss atomizer.

2. The spore suspension was injected into the florets by means of a hypodermic needle. The hypodermic needle method proved to be more efficient for producing infection but it is a very tedious and time-consuming one. It was therefore used mainly as a supplemental method to determine whether or not the inoculum was capable of producing the disease.

Temperature and humidity experiments were conducted in closed glass chambers, the pots of rye or other plants being held at the experimental conditions throughout the incubation period of the pathogen, that is, from the time of inoculation till the first signs of honeydew formation were evident. Most of the other experiments were also conducted in the closed chambers so that a high relative humidity could be maintained for at least part of the incubation period.

Ergot Infection and Stage of Floral Development

Brentzel (5) reported that a suspension of conidia sprayed onto rye heads in bloom produced droplets of honeydew in 7 to 10 days. No details of the exact stage of development or other conditions were given.

In 1902 Engelke, as reported by Atanasoff (2), found that infection with conidia was successful only before the flower was pollinated. At this stage the conidium can germinate in the nectar of the flower and produce a germ tube which can then extend into the cavity of the ovary. Stager, in 1903, obtained infection by artificial inoculation with honeydew when the plants were in full bloom. He also showed that plants inoculated 3 to 4 days before flowering became infected readily. In 1906, Tschermak observed that in rye, infection takes place in pollinated as well as in unpollinated flowers. The failure of flowers to become pollinated offers a greater chance for ergot infection both in rye and barley, as the glumes remain open for a longer time. Kirchoff (23) reported infection occurring from inoculations of rye at 5 to 6 days before flowering, at flowering, and up to 15 days after flowering. The abstract referred to did not describe his methods so that it is difficult to evaluate the significance of his results.

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Experimental

The rye or other grains sown in pots in the greenhouse did not all head and bloom at the same time. When most of the florets were in full bloom there were also some that ranged from pre-bloom to post-bloom stages.

The first experiment was begun on August 17, 1948, with rye which had been sown in the greenhouse early in May. Five plants representative of each of the following stages of development were selected and labelled:

(a) Pre-bloom - 3 to 5 days before any signs of flowering.
(b) Early bloom - only the first few anthers showing.
(c) Full bloom - florets wide open.
(d) Post-bloom - one week to 10 days after full bloom. The florets were often still wide open but the stamens were dried out and the ovaries considerably enlarged.

All the plants were sprayed at the same time with conidia suspended in a 1% maltose solution. Beginning in the afternoon of August 17, three sprayings were applied on successive days. The experiment was conducted under ordinary greenhouse conditions but with an attempt to maintain a high humidity by flooding the soil under the benches.

Honeydew formation was first observed on August 31, 14 days after the first spraying, and notes of sclerotial formation were taken on September 15.

Each labelled head was carefully examined and the number of sclerotia produced was recorded. The results are summarized in Table 5A. The experiment was repeated a month later. These results are shown in Table 5B.

Table 5

Infection of Rye at Different Stages of Floral Development

	Stage	Plant number 1	2	3	4	5	Total No. of sclerotia
A.	Pre-bloom	1	0	1	0	0	2
	Early bloom	0	5	2	0	0	7
	Full bloom	0	7	0	1	1	9
	Post-bloom	0	0	0	0	0	0
B.	Pre-bloom	1	2	0	0	0	3
	Early bloom	1	1	0	4	0	6
	Full bloom	0	0	5	1	0	6
	Post-bloom	0	0	0	0	0	0

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[illegible]

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Although infection was obviously not heavy in either of the experiments, the close agreement of results from both experiments indicates that stage of blooming at the time of inoculation did have some effect on whether or not infection took place.

No infection was observed in any of the plants which were inoculated after the flowering period was over and the ovaries had already become enlarged. Only a small amount of infection occurred when plants were inoculated before any external signs of flowering were evident. This might be accounted for by the closed condition of the glumes which prevented inoculum from coming in contact with the internal parts of the florets. It is possible that even with apparently closed glumes some of the tiny spores in a fine spray may have found their way into the floret and caused the small amount of infection which was reported. The most abundant infection occurred in the early and full bloom stages of development. If opening of glumes is the criterion for susceptibility to infection then certainly ideal conditions for infection were provided by the blooming rye plants in this experiment.

The results of these experiments indicate that, up until fertilization takes place, the susceptibility to infection by spray inoculation with ergot conidia is governed

by the condition of the glumes with respect to the extent to which they prevent entrance of inoculum to the internal floral parts. Once fertilization has taken place the inception of fertilization of the ovaries is apparently responsible for the lack of infection. The problem of finding out the true nature of this loss of susceptibility would be an interesting one for future study.

Varietal Resistance to Infection by the Ergot Fungus

Observations made on some of the University plots in the fall of 1948 showed a very heavy infection of ergot in the barley hybrids, especially on tillers which remained green late into the fall. Close examination revealed that, unlike the normally developing barley, the plants bearing heavily ergotized florets were characterized by glumes which remained permanently open. Their behaviour was similar to that of rye which exhibits the open glume condition because it depends on cross-pollination for fertilization. This observation suggested that varietal resistance to infection by the ergot fungus may be associated with the morphology, or

behavior of the glumes during flowering. In a discussion on varietal susceptibility in wheat, Weniger (44) stated that the Amber durum wheats are the most susceptible, the Red durum wheats are less susceptible, and the hard red spring wheats are seldom attacked. This she attributed to the relative difference in opening of glumes in these different kinds of wheat.

Experimental

Hybrid barley from the above mentioned stocks, Montcalm barley, and Newal barley, were grown in the greenhouse for inoculation studies. As far as is known, Newal is rarely affected by ergot but Montcalm has been reported to have been heavily infected on several occasions. Careful notes were taken on glume behaviour in the three lots of barley.

The glumes of some of the hybrid barley plants opened as the head emerged from the sheath and remained open for the entire period under observation, even after infection had taken place.

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The Montcalm barley emerged from the sheath with closed glumes but the tips of the glumes began to separate once the head was fully emerged and opened sufficiently to expose the organs in the upper part of the florets. This condition persisted for several days during the early to full flowering period but the glumes seemed to close again before development of the seed was evident.

No marked opening of the glumes was observed in the Newal barley. Florets had to be opened with forceps to determine the development of the stamens. Some heads only partly out of the sheath were in full bloom or even developing seed. To obtain heads in the pre-bloom stage they had to be withdrawn from unopened sheaths.

Inoculation was performed by the spray and hypodermic methods using a 1% maltose solution spore suspension. The plants were inoculated in the full bloom stage. At this time the hybrid florets were wide open, Montcalm florets were open just at the tip, and the Newal florets were closed. Four pots of each variety were used for inoculation. Two heads in each pot were labelled and inoculated with the hypodermic. All the heads were then sprayed with the spore suspension and placed in the humidity chamber at 20°C. Inoculation was repeated in the same manner on three successive days. On the tenth day after the first inoculation the pots were removed

and placed on greenhouse benches. Three of the hybrid plants already showed slight honeydew formation. Two weeks later the number of heads bearing sclerotia were recorded for each type of barley. The results are summarized in Table 6.

Table 6

Ergot Infection in Different Varieties of Barley

Variety	Heads infected	
	Sprayed	Hypodermic
Hybrid*	4 (45%)	4
Montcalm	3 (8%)	2
Newal	0	2

* Based on 9 lax-glume type heads.

Although the same number of pots was used for each variety and approximately the same number of plants came into head, the intended comparison of different glume types was not quite satisfactory because of the small percentage of lax-type heads developing in the hybrid barley. In all four

pots there were only 9 typical lax-type heads, and the remainder exhibited normal, tightly closed glumes at all times. The total population of Montcalm and Newal was at least 40 heads each. If the infections were compared on the percentage basis, the hybrids and Montcalm barleys would be rated as approximately 45% and 8% heads infected respectively.

The results of this experiment indicate that the nature of susceptibility to infection by the ergot fungus in the three types of barley studied is definitely associated with the opening of glumes during the flowering period. When the inoculum was introduced into the florets, in contact with the vulnerable parts, all three types of barley became infected. On the other hand, the spray method of inoculation which could not deposit the inoculum inside the floret when the glumes were tightly closed, as in the Newal barley, failed to produce infection. Montcalm, being intermediate in glume opening, was only slightly infected by this process.

Relative Susceptibility of Cereal Crops

One need not go far into the literature to realize that of all the common cereal crops, rye is the most susceptible host of the ergot fungus. Examination by Dillon Weston, and Taylor (11), of the records of ergot in cereals in England during the 24 years preceding 1942, indicated that the fungus occurred more commonly on rye than on any other crop; that it occurred rather frequently on wheat, less so on barley, and was very rarely found on oats. Moore (32), in a report on cereal diseases in England and Wales also stated that ergot occurs on rye, wheat, barley, and oats, in descending order of prevalence.

Duc (13) reported a severe attack of ergot in a field of wheat in the province of Ain in France. DeMonicault (10) referred to this same outbreak and suggested that the abrupt alternations of early heat, rain, and cold, induced an unusually open condition of the glumes so that the enclosed florets were more accessible to the attacks of the fungus. Stager (38) successfully inoculated wheat with a rye strain of C. purpurea by means of a hypodermic syringe, and explained the rare occurrence of ergot in wheat as the result of the

brief and irregular opening of the glumes at maturity, the susceptible parts of the flower being ordinarily protected by the glumes. In contrast to this behaviour in wheat, it is known that the flowers of rye, because of their cross-pollination habit, remain open and liable to infection for the entire flowering period.

Ergot on barley has been reported quite frequently. White (42) reported that in Tasmania, for the period 1943-45, Goldthorpe barley showed 5% infection with ergot. Neill (33) reported the common occurrence of ergot on six-rowed barley and its occasional occurrence on wheat and oats in New Zealand. Witte (43) in a report of the State Seed Testing Station in Stockholm, stated that ergot was detected in 53 and 24% respectively of rye and six-rowed barley samples submitted for inspection.

In contrast to the above observations, Ducellier (14) stated that C. purpurea is found in Algeria on many wild grasses but amongst cultivated cereals it has so far been observed only on oats. Rye appears to be free from the disease though the seed had been imported from localities in Europe and America where ergot of rye is common. No ergot has been found on wheat although infected oat plants were present within fields of wheat examined.

The first suggestion of biologic specificity in the ergot fungus was proposed by Stager in 1910 and still remains the main source of information regarding the host specialization of this pathogen. As reported by Weniger (44), Stager proposed the division of the species C. purpurea into three distinct biologic races:

1. The rye form, which infects most of the common cereal crops including oats.
2. A form which occurs on Brachypodium sylvaticum and has a very limited host range.
3. A form found on Lolium perenne and which also has a limited host range.

Stager's work has been severely criticized by some recent workers as being inadequate to justify the division of this species into the three forms proposed. The above mentioned report of Ducellier suggests that either Stager's lists of hosts attacked by the different forms are in error, or that he has failed to report a form which attacks oats but does not attack wheat or rye.

The present study does not contribute much to the knowledge of physiologic specialization in the ergot fungus but infection experiments were carried out to learn something of the comparative effect of the pathogen on the commonly grown cereal crops.

Experimental

Red Bobs wheat, Victory oats, Montcalm barley, and spring rye were used in this study. As the plants came into the flowering stage they were inoculated with a 1% maltose spore suspension of C. purpurea and incubated in humidity chambers.

Inoculations were performed by both the spray and hypodermic syringe method. Four pots of each crop were selected for inoculation. Two heads in each pot were inoculated with the hypodermic and labelled. All the plants were then sprayed with the spore suspension and left in the humidity chambersfor two weeks. They were then removed from the humidity chambers and left in the greenhouse for further development.

Notes were taken on the condition of the glumes and stage of floral development at the time of inoculation for each of the crops under test. An attempt was made to inoculate each of the crops at approximately the same stage of floral development. This was achieved by inspecting the florets at frequent intervals until most of them showed some pollen formation. Florets of oats and wheat had to be opened with a pair of forceps to facilitate such observations. At the time of inoculation the oats and wheat florets were

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completely closed; the barley florets were partly open at the tips; and most of those in rye were open wide enough to expose to view most of the floral parts.

When the plants were removed from the humidity chambers only the rye showed honeydew formation. Some of the barley florets which had been inoculated with the hypodermic, showed honeydew two days later. No honeydew was observed in wheat or oats. Final inspections and notes on sclerotial formation were taken on January 24, two weeks after removal of the plants from the humidity chambers. The number of heads bearing slcerotia were recorded for each crop. The results are summarized in Table 7.

Table 7

Relative Infection in the Common Cereal Crops

Crop	Number of heads infected		
	Spray	Hypodermic	Total
Rye	26	6	32
Barley	2	5	7
Wheat	0	2	2
Oats	0	2*	2

* Infection not definite.

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The results shown in the table leave no doubt as to which crop was most easily infected by the fungus. Rye was by far the most susceptible. Barley, wheat and oats followed far behind in order of descending susceptibility. The suspected infection in the oats was by no means definite. The sclerotia-like bodies within the florets were very small, and only an abundant layer of conidia on this small, darkened body led to the belief that the fungus must have become established.

The lack of infection in wheat and oats which had been sprayed with the conidial suspension, and the presence of slight infection occurring in florets which had been inoculated with the hypodermic syringe, indicates that the resistant properties of these crops are associated with the exclusion of inoculum from susceptible parts rather than with genetic characters of resistance within the ovary. On the other hand, the lack of conclusive evidence for infection in oats suggests something in the nature of defensive reaction by the host or biologic specialization of the pathogen.

Moisture and Temperature Effects

Although moisture and temperatures are distinctly different factors of the environment their effect on the development of certain diseases, such as ergot of cereal crops, is related to such an extent that the one is seldom discussed without consideration of the other. For this reason they have been studied together here as the major environmental factors influencing inception of disease.

The literature on the influence of these factors on infection by the ergot fungus is mostly of a general nature, most of the observations being made in the field with no attempts being made to control conditions. Vladimirsky (40), in a report on the geographical distribution and zones of injurious influence of ergot on rye in the U.S.S.R., stated that the greatest development of ergot infection was observed in regions where the following conditions prevailed:

1. A relative humidity during flowering of not less than 70%.
2. A temperature at the beginning of flowering not above 15°C.
3. A flowering period of 13 days.

He was able to divide regions into three groups according to meteorological conditions considered to be optimal, intermediate, and adverse for the development of ergot and found these to roughly coincide with the regions of maximum, medium, and minimum importance of the disease. Hynes (21), working on methods of producing ergot in New South Wales, confirmed the above mentioned observations by concluding that only in cooler, moister climates can field cultivation of ergot be considered. In 1947, Hershenson and Plaut (20) reported on some experiments carried out in Palestine and they made several observations regarding the inoculation of rye with C. purpurea as related to environmental conditions. They concluded that in Palestine the days are usually too dry for infection to take place so that it occurs mainly at night when the humidity rises to about 80% from the daytime level of around 30%. They also stated that a temperature of at least 15° C. is required so that in cooler climates the temperatures may be too low at night but that the humidity is usually high enough during the day.

The studies reported here are of a more specific nature, the work having been done in the greenhouse under controlled conditions of temperature and humidity.

Materials and Methods

Spring rye grown in 8-inch pots was used throughout. Inoculum consisted of 1% maltose suspension of conidia harvested from test-tube cultures of C. purpurea.

Three glassed-in chambers, 4 feet by 4 feet, and 4½ feet high, were available for this work in the greenhouse. These were provided with thermostatically controlled heating units and one of them was also equipped with cooling facilities. Depending on the temperature of the greenhouse, a fairly accurate control of temperature could be maintained in these chambers over the range from about 5°C., to 35°C. or more. During hot summer days, when the temperature in the greenhouse rose to 25°C. or higher, it was impossible to maintain intermediate temperature levels because only one of the chambers had cooling facilities. Most of the work was therefore done during the cooler seasons of the year.

Experiments were conducted at three controlled levels, namely: approximately 50%, 80 - 90%, and 100%. The relative humidity of the atmosphere in the greenhouse was usually around 50% as measured by a sling psychrometer. It was estimated that the relative humidity of the controlled temperature chambers provided with a large pan of water was approximately 80 - 90%. One hundred per cent relative humidity

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was obtained by means of a Westinghouse Vital-Air humidifyer which produced a blast of mist or fog when in operation. This apparatus, operated for about $\frac{1}{2}$ hour every 10 or 12 hours, maintained a constant film of condensed moisture on all the inside surfaces of the chamber so that it was apparent that the atmosphere within the chamber was saturated.

The usual spray method of inoculation was employed using the conventional type of atomizer manufactured by the DeVilbiss Co.

Experimental

Since an experimental setup to study the complete temperature-moisture relationship with respect to infection would need to be very extensive, and facilities were limited to three controllable conditions at one time, the study was divided into experiments on temperature separate from those on humidity. Experimental results will therefore be reported on separately also.

Temperature

In the first experiment infection was studied at 10°, 20°, and 30°C., and in a second at 15°, 25°, and 35°C.

Twelve pots of rye plants in the mid-flowering stage of development were inoculated by spraying with the conidial suspension. Four pots were then placed in each of the 3 chambers controlled at temperatures of 10°, 20°, and 30° C. respectively. A relatively high humidity was maintained in the chambers by providing a large pan of water in each chamber. Inoculations were repeated on three successive days. The pots were left in the chambers for 3 weeks after which time they were removed and examined for development of ergot sclerotia. A similar procedure was followed in the second experiment using temperatures of 15°, 25°, and 35°C.

Sclerotial development was quite distinct when the plants were removed from the chambers. The number of heads infected and the number of sclerotia per head were recorded. The combined results of both of the experiments are summarized in Table 8. They represent the total infected heads and total number of sclerotia for each of the temperatures tested.

Table 8

Effect of Temperature on Ergot Infection of Rye

Temperature °C.		Number of heads infected	Total number of sclerotia
1.	10	3	3
2.	15	9	23
1.	20	9	16
2.	25	8	14
1.	30	4	6
2.	35	0	0

1. First experiment.

2. Second experiment (conducted one month later).

Since no notes were taken at the beginning of the experiments on the number of heads in the susceptible stage the results cannot be expressed in percentage of heads infected. Nevertheless, the data as recorded is considered representative in spite of the uneven flowering of the plants because this was common to all the replicates and although many of the uninfected heads may not have been in the

susceptible stage at the time of inoculation, the same would be true for all the other treatments in the experiment.

The records show that the best infection occurred at 15°, 20°, and 25°C., but that a single optimum temperature cannot be determined from the results of these experiments. Little infection occurred at 10° and 30°C., and none at 35°C.

The infection results do not agree very closely with those on spore germination. While germination of conidia was low at 15°C. when compared to that at higher temperatures, infection at 15°C. was as good or better than that at 20° and 25°C. It is possible that, with abundant inoculum, high germination percentage is not necessary for abundant infection. On the other hand, notes on spore germination were taken at the end of 48 hours but when cultures were left for 96 hours, germination percentage at 15°C. increased considerably. Such may have been the case in the infection experiments. The conidia were given sufficient time in a favorable habitat to permit good germination in spite of the comparatively low temperature.

Moisture (Humidity)

For the controlled humidity experiments approximate humidities of 50%, 80 - 90%, and 100% were maintained in the

Susceptible stage at the time of inoculation, the same would be true for all the other treatments in the experiment.

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Moisture (Humidity)

For the controlled humidity experiments approximate humidities of 50%, 60 - 90%, and 100% were maintained in the

closed-in glass chambers. For a uniform temperature in all three chambers the heating and cooling fixtures were shut off so that the temperature condition was that of the greenhouse as a whole. This was approximately 20°C. The experiment was set up in a manner similar to that in the temperature experiments, except that the plants were removed from the chambers one week after inoculation and allowed to develop for 2 weeks on the greenhouse bench before infection notes were taken. The number of heads infected and the number of sclerotia per head were recorded. The results are summarized in Table 9.

Table 9

Effect of Relative Humidity on Ergot Infection of Rye

Relative humidity	Number of heads infected	Total number of sclerotia
50%	5	7
80 - 90%	13	25
100%	11	29

There seems to be no difference between the number of heads infected at 80 - 90% relative humidity and that at 100%, but the infection at these two levels of humidity does appear to be significantly higher than that at 50%. One difference that did seem to exist between infections at the two higher levels of humidity was the number of florets infected per head. At the 80 - 90% relative humidity a total of 25 sclerotia were formed in 13 heads infected while 29 sclerotia were formed in 11 heads infected at the 100% level. This is an average of 1.92 sclerotia per head in the former as compared with 2.64 in the latter.

The higher infection ratio for the 100% relative humidity might be associated with the frequent condensation of moisture on the surface of the florets. These droplets of condensed moisture may have aided in the deposition of initial inoculum at the base of the ovary thus increasing the chances of infection once the spores did germinate, or this condensed moisture may have merely provided a better medium for the germination of the spores. Another possibility is that, once infection has set in, conidia produced by this initial infection may be transported, by these droplets of water, to florets below the infected ones and thus aid in infecting a greater number of florets per head.

Discussion

As in the case of spore germination, no previous reports on controlled studies of infection by the conidia of C. purpurea were found in the literature. Numerous references have been made to field observations but no attempts to study infection under controlled conditions have been reported.

Experiments which were made to determine the relationship of the stage of floral development and ergot infection showed that the rye floret remains susceptible to infection for a relatively long period of time but not nearly as long as was reported by Kirchoff (23). He stated that infection occurred from inoculations of rye at 5 to 6 days before flowering, at flowering, and up to 15 days after flowering. Depending upon when he considered the flowering period to be terminated, the period of susceptibility suggested by him extends at least one week beyond that indicated by the results of the present study. Otherwise, the present study agrees with his observations. The fact that the present results were not obtained under natural conditions may account for the difference.

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The relative susceptibility of different cereal crops to infection by the ergot fungus was found to be as follows: rye, barley, wheat, and oats, in order of descending susceptibility. It was found that all the crops tested, except probably oats, can be readily infected by the rye strain of the ergot fungus but that the difference in susceptibility was associated with the difference in opening of glumes. Varietal resistance was accounted for on the same basis. The open glume types of barley found in hybrid stocks were readily infected by spraying with conidial suspension during flowering. Montcalm was only slightly infected, and no infection occurred in Newal which exhibited tightly closed glumes at the time of inoculation.

Results on temperature and humidity effects on infection agree quite well with the field observations made by Hershenson and Plaut (20). They claimed that the optimum temperature range for infection of rye in Palestine was 20° to 30°C. and that infection was best when the relative humidity was more than 70%. Present studies showed that the optimum temperature range was 15° to 25°C. and that infection at relative humidities above 80% was much higher than that at 50%.

GENERAL DISCUSSION

The present study has contributed to the understanding of several aspects of the development of the ergot fungus. First of all, it has provided further evidence of the importance of environment as a controlling factor in the establishment and development of the disease. The observations with regards to the influence of moisture and temperature serve as good examples of this type of evidence. As has been mentioned before, these two factors are very closely associated in their function in nature and are often the most important factors that determine the activity of a pathogen. Judging from the results of the experiments here, one would certainly expect the ergot fungus to function most efficiently in moist, temperate climates. The conidial stage, which is considered to be most important in the production of epiphytotics, is subject to considerable limitation by the influence of these two factors. Conidia will not germinate at temperatures much below 10°C. nor much above 30°C. and are permanently inactivated at 35°C. Moisture requirements likewise are quite definite. It was found that conidia require a liquid medium in which to germinate and will not germinate on dry surfaces

even in an atmosphere of 100% relative humidity. Inoculation experiments showed that very little infection occurs in an atmosphere of 50% relative humidity even if all other factors are favorable. Bearing in mind the moisture requirement for germination of conidia it is understandable why the latter relationship exists. Only with a high relative humidity is there any possibility for condensation of moisture to occur and provide the liquid medium necessary for the conidia to germinate. Moisture in the form of rain may often be harmful to the pathogen because it would tend to wash the spore-laden honeydew away from the susceptible parts of the plant.

An important observation in the present study was that dilution of honeydew is necessary before any conidia suspended in it can germinate. Honeydew may be transported by insects and other vectors to susceptible parts of numerous plants but unless sufficient moisture is available to dilute this suspension of spores, infection cannot occur. Furthermore, the timing of these conditions is also important. The period in which plants are susceptible to ergot infection is rather limited as compared to that for several other cereal diseases. If environmental conditions are not favorable during the 2-3 week flowering period, there is little likelihood of much infection occurring.

The stimulatory action of sugars on germination is interesting from the standpoint of similar functions of the honeydew in nature. In addition to preventing germination of conidia until such a time as conditions are favorable for the development of the fungus the honeydew likely stimulates rapid germination so as to take advantage of favorable conditions as soon as they are provided. Furthermore, the observation that some sugars are better than others in stimulating germination provides information for more detailed studies of nutritive relationships in the germination of such conidia.

With respect to the application of the information obtained, for the purpose of developing effective methods of testing ergot resistance in crops, there are several factors which deserve consideration at this stage:

1. That, as far as could be determined from the literature and from the experimental work in the present study, the opening of glumes during flowering is the factor which is most likely to determine the extent to which a crop may become infected by the ergot fungus. Apart from the fact that physiologic specialization of the pathogen has presumably been demonstrated, no deep-seated character of resistance has yet been demonstrated in the host.

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2. If an effort to investigate the possibility of resistance due to deep-seated, genetic factors is to be made, it is necessary to develop a method of inoculation which will not be interfered with by the difference in opening of the glumes. One of the methods employed in the present study, that of injecting inoculum by means of a hypodermic syringe, largely overcame this glume factor but this method involves a very tedious and laborous procedure and would not likely be feasible on a large scale. It has been suggested that an apparatus used in testing selections of wheat and barley for reaction to loose smuts might be adapted for this purpose. This apparatus is described by Moore (32) and employs the vacuum principle of introducing the inoculum into the florets. The present writer has not had an opportunity to use such an apparatus but the principle appears promising and it is hoped that a suitable adaptation of it may be developed before long.

3. That discovery of genetic resistance to ergot would be an important step in obtaining control of the disease even if some crops and varieties are presumably resistant because of the typically closed nature of their glumes during flowering. Opening and closing of glumes is greatly influenced by environmental conditions and, as illustrated

by DeMonicault (10), may be responsible for epiphytotics in crops which are ordinarily very seldom affected by the disease. The occurrence of ergot on wheat, usually regarded as quite exceptional, was very frequent in France in 1922. DeMonicault considered this to have been caused by the abrupt alternations of early heat, rain, and cold which induced an unusually open condition of the glumes, so that the enclosed florets were more susceptible to attacks of the fungus. Such irregular outbreaks might be avoided by the development of genetically resistant varieties.

SUMMARY

1. The influence of external environmental factors on the ergot fungus, Claviceps purpurea, and on infection of plants by it was studied in this investigation with the main purpose in mind of improving techniques of testing the varietal resistance of cereal crops.

2. The studies were confined to the conidial stage of C. purpurea; to factors influencing germination of the conidia and to infection of cereals by this stage.

3. The minimum, optimum, and maximum temperatures for germination of the conidia were determined. These were: 5° to 10°C., 20° to 25°C., and 30° to 35°C. respectively. It was further found that conidia survived storage in ice at approximately 0°C. but were killed by prolonged exposure to a temperature of 35°C.

4. Water in liquid form is required for germination. Conidia on dry glass cover slips exposed to humid atmospheres for 72 hours were rendered ungerminable.

5. Sugars, and particularly maltose, were found to stimulate germination considerably.

6. Only the lower concentrations of maltose were stimulatory. Inhibitory effects were evident at concentrations greater than 0.1 molar and no germination occurred at concentrations above 0.5 molar.

It was observed that considerable dilution of the honeydew, the sugary medium in which the conidia are produced in nature, is necessary before germination of these spores will take place.

7. It was shown that germination occurred equally well in continuous light, in continuous dark, and in alternating light and dark.

8. Germination was fair in redistilled water at pH levels from 4.0 to 9.0 but no germination occurred at pH 3.0 and considerable inhibition was evident at pH 10.0

9. Rye florets were readily infected shortly before flowering and at stages up to late flowering, a period of about 3 weeks under greenhouse conditions.

10. Inoculation studies showed that the commonly grown cereal crops became infected as follows: rye, barley, wheat, and oats, in decreasing order of susceptibility.

11. The susceptibility of different varieties of barley was shown to be associated with the opening of glumes during flowering. Hybrid barley, exhibiting widely opened glumes, became readily infected; Montcalm, with intermediate glume opening, was only slightly infected; and Newal, with closed glumes, did not develop the disease.

12. The most favorable temperatures for infection following inoculation with conidia were found to be between 15° and 25°C. Little infection occurred at 10° and 30°C., and none at 35°C.

13. Relative humidities of 80% and higher favored infection. No differences in degree of infection were observed under conditions of 80% and 100% relative humidity but infection was considerably reduced at the 50% level.

14. A vacuum method of inoculation is suggested for testing ergot resistance in further studies.

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